International Journal of Agricultural
Science and Research (IJASR)
ISSN(P): 2250-0057; ISSN(E): 2321-0087
Vol. 6, Issue 2, Apr 2016, 29-34

TRANS STELLAR
•Journal Publications •Research Consultancy

ISOLATION OF *ESCHERICHIA COLI* FROM DIARRHEA AND TEST THEIR PATHOGENSITY AND SUSCEPTIBILITY PATTERN FOR ANTIBIOTIC

RABBAB JABBAR SAKHI

Departments of Animal Resources, College of Agriculture, Sumer University, Iraq

ABSTRACT

This study was design to isolation and biochemical identification of Escherichia coli from diarrhea in children, test susceptibility pattern for antibiotic and pathogenesis of Escherichia coli. For these reason 150 samples of stool gathered from patients children in different region of Dhi-Qar city in Iraq for the period Jaunuary 2015 to June of the same year. Results of the bacterial growth showed that 111 samples were positive bacterial growth with percentage (74%) , isolate were microscopically and biochemically examined and diagnosed by Api 20E kit, The results showed 84 isolate belong to Escherichia coli with percentage 75.6% .

The sensitivity of isolates were examined for antibiotics, the isolates showed a height resistance for Ampicillin, while the others showed highest sensitivity for Co – trimoxazol ,Erythromycin , Chloramphenicol.

The study showed the ability of Escherichia coli to elicit an inflammatory response in mouse intestine after experimental infection that induced by orally dosing with Escherichia coli.

KEYWORDS: Enteropathogenic E. Coli, Pathogensis, Antibiotic Resistance

Received: Feb 04, 2016; **Accepted:** Feb 12, 2016; **Published:** Feb 20, 2016; **Paper Id.:** IJASRAPR20165

Original Article

INTRODUCTION

Enteropathogenic *Escherichia coli* (EPEC) is a human enteric pathogen that attaches to the surface of intestinal epithelial cells and causes watery diarrhea (Moon *et al*.,1983). *Escherichia coli* is one of the most common causes of morbidity and mortality in children with diarrhea all over world particularly in developing countries (Enayat *et al*.,2011). Diarrheal diseases continue to be a health problem worldwide. (Passariello *et al*.,2010; Kosek .,2003).

For most patients, the illness is a self-limited one. But, disease can cause severe fluids and electrolytes loss, which require prompt treatment. The management of acute diarrhea is based on replacement of fluids. However, antibiotic might be required for the management of the same cases and may reduce the duration of disease, but use is restricted due to emergence of resistance or due to lack of availability in some countries (Phavichitr and Catto-Smith., 2003). Antibiotic therapy in hospitals is possibly the most important factor that increases antibiotic-resistant microorganisms (Tacconelli *et al* ., 2009) . The emergence, propagation, accumulation, and maintenance of antimicrobial resistant pathogenic bacteria have become significant health concerns, and lead to increased morbidity, mortality, and health-care costs as a result of treatment failures and longer hospital stays (Levy and Marshall.,2004; Salma.,2008).

Despite progress made during the last decade regarding the study of EPEC pathogenesis, relatively little is known about EPEC-induced physiological changes. In order to adequately define these changes, an animal model is needed. Animal models have been used to study host responses to EPEC homologues; these models include rabbits infected with rabbit REPEC (Abe *et al* .,1998. ; Tauschek *et al*.,2002; Vallance and Finlay., 2000) there are limitations to the use of this model, such as a paucity of genetic and immunological resource(Abe *et al* .,1998). Mouse models have the advantage of allowing the use of genetically modified animals for further studies.

MATERIALS AND METHODS

Sample Collection

A total of 150 diarrheal faecal samples from the patients children affected with diarrhea were collected in different region Dhi-Qar, Iraq, during January 2015 – June 2015.

Isolation of *E.coli*

A swab of faecal sample was cultured directly on MacConkey agar, Salmonella–Shigella agar(SSA),Eosin methylene blue(EMB) ,blood agar. Petri dishes were kept in the incubator for 24 hours at 37ºC (Hajna and Perry, 1939). After 24 hours, The plates were examined and studied carefully for the presence of characteristic colonies of *E.coli* . Microorganisms grown on MacConkey agar are capable of metabolizing lactose which produces acid by-products that lower the pH of the media which causes the neutral red indicator to turn red, and if sufficient acid is produced, a zone of precipitated bile develops around the colony (Koneman, 2005). Different biochemical tests (Werkman, 1930; O'Meara, 1931; Vaughn *et al*., 1939; Silva *et al*., 1980) were performed for the identification of *E. coli* (Table 1). Api 20E kit (biomeriux , france) also used for identification of the bacteria.

Table 1: Result of Biochemical Test of *E. coli*

Indole Test	Methyl Red Test	Voges Proskeur Test	Simmon's Test	
			Ammonium Acetate Test	Ammonium Citratetest
+	+	-	+	-

Antibiotic Susceptibility Test

All E. coli isolates were tested for their susceptibility toward Gentamycin, Rifampicin, Ampicillin, Tetracycline, Co-trimoxazol, Nitrofuranation, Chloramphenicol and Cefotaxime following the procedure of Bauer et al .,Similar colonies from pure culture, transferred to nutrient broth incubated at 37^{o}C for 18-24 hours, then centrifuged, a suspension was prepared by adding a normal saline adjusted to McFerland Opacity Standard tube number(1), 0.1 ml from bacterial suspension was inoculated on to Muller-Hinton agar with the swab in such a way that the whole surface of agar was coverd by a dry cotton wool swab. The antibiotic disks were dispended on the surface of the medium by sterilized forceps and incubated at 37^{o}C for 24 hours. The results was recorded as resistant or susceptible by measuring of the inhibition zone diameter in milliliter. (Bauer et al., 1966).

Experimental Design (Pathogenesis Study)

There were two groups of mice ,each group contained three mice, first group Mice were infected with EPEC in dose about 0.25ml/mice which contained 1.5× 10^{8} for 7 days the second group injected with(0.25 ml) phosphate puffer saline ,the animals were observed daily for activity level and water intake, and weight was measured.

At various times following infection, animals were sacrificed, and intestinal tissues were processed for further analysis.

RESULTS AND DISCUSSIONS

Isolation of *E. coli*

According to the cultural, microscopical, biochemical in addition to Api 20E kit Figure 1, One hundred and eleven specimens were positive for bacterial culture. E. coli formed 75.6% of total positive specimens.

The dominance with these percentage is because of *E.coli* have many virulence factors such as Attachment and Effacing Factor (AEF) and Fimbrial Adherence Factor (FAF) which make these bacteria able to attached to epithelial layer of intestine ,the E.coli also able to produce enterotoxins Beside the *E.coli* belong to *Enterobacteriaceae* which is live as harmless commensals in animal intestines (Qadri et al.,2000).

Figure 1: Result of API 20 System of EPEC

Antibiotic Susceptibility

Results demonstrate that all E. coli isolates were resistant to ampicillin with percentage 100% ; while Erthromycin recorded the lowest resistance percentage (14.1%) Table 2

Table 2: Comparison of Nine Antibiotic Resistance Patterns of the E. *coli* Strains Isolated from Patients Children with Diarrhea

Antimicrobial Agent	No. of Isolate		Rate of Resistant %
	Resistant	Non- Resistant	
Gentamycin	36	48	42.8
Rifampicin	49	35	57.1
Ampicillin	84	0	100
Tetracycline	66	18	78.57
Co-trimoxazol	20	64	23.8
Nitrofuranation	33	81	39.2
Chloramphenicol	14	70	16.6
Erthromycin	12	72	14.2
Cefotaxime	72	12	85.7

Accurate use of antimicrobials may be beneficial in preserving antimicrobial efficacy and substantially reducing diarrheal illness. However, antibiotic therapy can further increase drug resistance in microorganisms (Tacconelli *et al*.,2009). In this study, we examined antimicrobial resistance of *E. coli* isolates from diarrhea. The highest levels of resistance were observed against Ampicillin and Tetracycline for pathogenic *E. coli*, which may be caused by the frequent use of these

antibiotics and the transfer of plasmids between bacteria (Roberts, 2003; Uma, 2009). In the *Enterobacteriaceae*, resistance to Ampicillin is mainly because of ß-lactamases (Kliebe, et al1985). Tetracycline resistance in bacteria is mediated by four mechanisms: efflux, ribosomal protection, enzymatic inactivation, and target modification (Chopra and Roberts,2001) .

PATHOGENESIS STUDY

Macroscopical Examination

To define host responses to EPEC infection, the mouse intestines was examined . The colon of uninfected mice contained formed pellets of stool beginning just distal to the cecum. However, the proximal colon of animals infected with EPEC for 10 days contained semisolid stool, and formed stool pellets were not seen until the distal colon also,the cecum appeared to be slightly engorged in EPEC infection.

Histopathological Examination

The colon of control animals revealed sparse intraepithelial lymphocytes (IELs) and lamina propria polymorphonuclear leukocytes (PMNs), consistent with the normal mucosal histology of conventionally housed mice. In contrast,the numbers of both IELs and lamina propria PMNs were significantly increased in the colon of EPEC-infected mice figure 2. Because intestinal inflammation has been linked to increased goblet cell differentiation (Ciacci et al,2002;Conour et al.,2002;Seto et al., 2003;Surawicz et al.,1994). EPEC infection also caused a significant increase in the number of goblet cells. In contrast, acute inflammation, as evident from intraepithelial PMNs and occasional crypt abscesses, occurred in a patchy distribution in the intestine of EPEC-infected mice and was not present in the intestine of control mice . Together, these data show that EPEC elicits an inflammatory response in the mouse intestine.

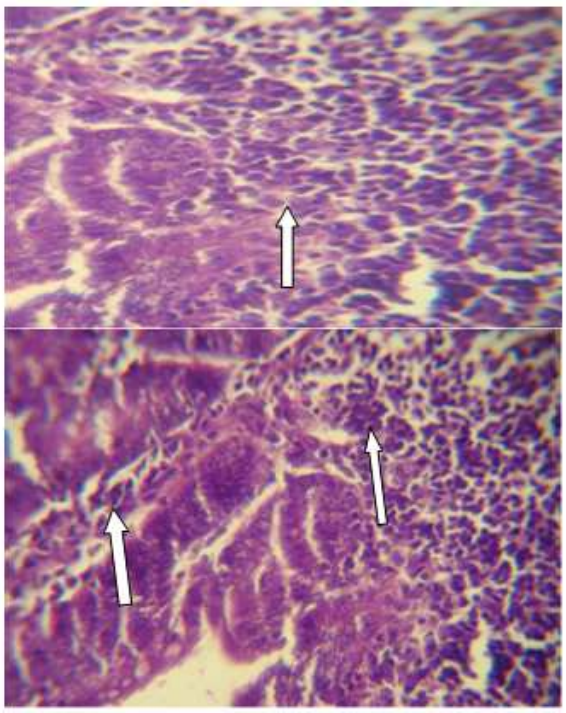

Figure 2: Histological Section in Intestine of Mice Infected with EBEC Both Iels and Lamina Propria PMNs Were Significantly Increased in Epithelial Lining of Intestine ← (H&E 40 X)

CONCLUSIONS

- *E. coli* is considered as one of the most important pathogen that cause diarrhea when isolated with percentage 75.6% from patient children with diarrhea from different region in Dhi-Qar city in Iraq.
- All isolate are resistant to while the is most significant treatment in the inhibition growth of *E.coli* .
- The pathogenesis test showed the ability of *E.coli* to elicit an inflammatory response when bacteria caused a histopathalogical change in mice intestine.

REFERENCES

1. *Abe, A., U. Heczko, R. G. Hegele, and B. B. Finlay. 1998. Two enteropathogenic Escherichia coli type III secreted proteins, EspA and EspB, are virulence factors. J. Exp. Med. 188:1907–1916. Although REPEC-induced disease in rabbits is similar to EPEC-induced disease.*
2. *Bauer, A. W., Kirby , W. M. , Sherris , J. C. and Turch, M. (1966). Antibiotic susceptibility testing by astandardized single disk method . Am. J. Clin. Pathol. 36(3):493-496.*
3. *Chopra I, Roberts M. Tetracycline antibiotics: mode of action, applications, molecular biology, and epidemiology of bacterial resistance. Microbiol Mol Biol Rev 2001 Jun;65(2):232e60.*
4. *Ciacci, C., D. Di Vizio, R. Seth, G. Insabato, G. Mazzacca, D. K. Podolsky, and Y. R. Mahida. 2002. Selective reduction of intestinal trefoil factor in untreated coeliac disease. Clin. Exp. Immunol. 130:526-531.*
5. *Conour, J. E., D. Ganessunker, K. A. Tappenden, S. M. Donovan, and H. R. Gaskins. 2002. Acidomucin goblet cell expansion induced by parenteral nutrition in the small intestine of piglets. Am. J. Physiol.283:G1185-G1196.*
6. *Davies J, Wright GD. Bacterial resistance to aminoglycoside antibiotics. Trends Microbiol 1997 Jun;5(6):234e40.*
7. *Enayat , Fariborz , Heiman , Mohammad . and Mehdi, (2011). Frequency, antimicrobial susceptibility and plasmid profiles of Escherichia coli pathotypes obtained from children with acute diarrhea. JJM. 4(1): 23-28.*
8. *Kliebe C, Nies BA, Meyer JF, et al. Evolution of plasmid-coded resistance to broad-spectrum cephalosporins. Antimicrob Agents Chemother 1985 Aug;28(2):302e7.*
9. *Koneman EW. Color Atlas and Textbook of Diagnostic Microbiology, Lippincott, JB. (Ed), Philadelphia. 313-317, 2005.*
10. *Kosek M, Bern C, Guerrant RL. The global burden of diarrhoeal disease, as estimated from studies published between 1992 and 2000. Bull World Health Organ 2003;81(3):197e204.*
11. *Levy SB, Marshall B. Antibacterial resistance worldwide: causes, challenges and responses. Nat Med 2004 Dec;10(Suppl. 12):122e9.Microbiol. Rev. 11:142–201.*
12. *Moon, H. W., S. C. Whipp, R. A. Argenzio, M. M. Levine, and R. A. Giannella. 1983. Attaching and effacing activities of rabbit and human enteropathogenic Escherichia coli in pig and rabbit intestines. Infect. Immun. 41:1340–1351.*
13. *Nataro, J. P., and J. B. Kaper. 1998. Diarrheagenic Escherichia coli. Clin. Microbiol. Rev. 11:142–201.*
14. *O'Meara RAQ. A simple delicate and rapid method of detecting the formation of acetylmethylcarbinol by bacteria fermenting carbohydrate. J Pathol Bacteriol. 34: 401-406, 1931.*
15. *Passariello A, Terrin G, Baldassarre ME, et al. Diarrhea in neonatal intensive care unit. World J Gastroenterol 2010 Jun 7;16(21): 2664e8.*

16. *Phavichitr N, Catto-Smith A. Acute gastroenteritis in children: what role for antibacterials?. Paediatr. Drugs. 2003; 5(5):279–290.*

17. *Qadri,F.,Das,S.K.,Faruque A.S.,Fuchs G.J.,Albert M.J.,Sack R.B.,and SvennerhKolm A.M.(2000) .Prevalence of toxin types and colonization factors in enterotoxigenic E. coli isolated during a 2-year period from diarrheal patients in Bangladesh .J.Clin.Microbiol. 38:27-31.*

18. *Roberts MC. Tetracycline therapy: update. Clin Infect Dis 2003 Feb 15;36(4):462e7.*

19. *Salma TG. Gram-negative antibiotic resistance: there is a price to pay. Crit Care 2008;12(Suppl. 4):S4.*

20. *Seto, Y., H. Nakajima, A. Suto, K. Shimoda, Y. Saito, K. I. Nakayama, and I. Iwamoto. 2003. Enhanced Th2 cell-mediated allergic inflammation in Tyk2-deficient mice. J. Immunol. 170:1077-1083.*

21. *Silva RM, Toledo MR, Trabulsi LR. Biochemical and cultural characteristics of invasive Escherichia coli. J Clin Microbiol. 11: 441- 444, 1980.*

22. *Surawicz, C. M., R. C. Haggitt, M. Husseman, and L. V. McFarland. 1994. Mucosal biopsy diagnosis of colitis: acute self-limited colitis and idiopathic inflammatory bowel disease. Gastroenterology 107 :755- 763.*

23. *Tacconelli E, De Angelis G, Cataldo MA, et al. Antibiotic usage and risk of colonization and infection with antibiotic-resistant bacteria: a hospital population-based study. Antimicrob Agents Chemother 2009 Oct;53(10):4264e9.*

24. *Tauschek, M., R. A. Strugnell, and R. M. Robins-Browne. 2002. Characterization and evidence of mobilization of the LEE pathogenicity island of rabbit-specific strains of enteropathogenic Escherichia coli. Mol. Microbiol. 44:1533–1550.*

25. *Uma B, Prabhakar K, Rajendran S, et al. Antibiotic sensitivity and plasmid profiles of Escherichia coli isolated from pediatric diarrhea. J Glob Infect Dis 2009 Jul;1(2):107e10.*

26. *Vallance, B. A., and B. B. Finlay. 2000. Exploitation of host cells by enteropathogenic Escherichia coli. Proc. Natl. Acad. Sci. USA 97:8799–8806.*

27. *Vaughn RH, Mitchell, NB, Levine, M. The Voges- Proskauer and methyl red reactions in the coliaerogenes group. J Am Water Works Assoc. 31: 993-1001, 1939.*

28. *Werkman CH. An improved technic for the Voges- Proskauer test. J Bacteriol. 20: 121-125, 1930.*